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INTERBREEDING AND DNA ANALYSIS OF SIBLING SPECIES WITHIN
THE Bactrocera dorsalis COMPLEX

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INTRODUCTION

The Bactrocera dorsalis complex consists of fifty-two species, of which, eight are known as serious
pests of major economic importance (Drew and Hancock 1994). Among the pest species, four
(namely B. carambolae, B. dorsalis, B. papayae and B. philippinensis) have been observed to
interbreed in the laboratory. B. carambolae interbreeds with B. dorsalis (McInnis et al. 1999) and
with B. papayae (Wee and Tan 2000); and B. dorsalis with B. papayae (Tan 2000a). Sexual
compatibility was also observed between wild B. dorsalis and wild B. philippinensis (Medina et al.
1998).

For species not included in the B. dorsalis complex, two sympatric species in eastern Australia - B.
tryoni and B. neohumeralis, were able to interbreed and produce viable hybrid progeny in the
laboratory. The hybrid could be found in the field (Gibbs 1968). In addition, interbreeding between
B. jarvisi and B. tryoni resulting in fertile F2 offspring has been conducted in laboratory experiments.
Males of the former species are not attracted to any known lures, while those of the latter are
attracted to cue lure. Both species are major pest species of commercial fruit crops in eastern
Australia. The F1 and F2 hybrids were morphologically distinct from their B. tryoni and B. jarvisi
parents (Cruickshank et al. 2001). Since not much research on interbreeding has been conducted on
Bactrocera species especially between sibling species within a complex, one of the aims of this
paper is to discuss recent studies on interbreeding of sibling species within the B. dorsalis complex.

Several DNA analyses have yielded accurate and valuable genetic information for studies of intra-
specific population structure and also for systematic relationships or genetic variation among species.
DNA analytical techniques based on the polymerase chain reaction (PCR) are useful as they can be
applied using small quantities (in nanograms) of genetic material from fruit flies (He and Haymer
1997). The other aim of this lecture, as requested by the organizers, is to present results of
studies/surveys based on DNA analysis of fruit flies using a) random amplification of polymorphic
DNA (RAPD)-PCR, b) restriction fragment length polymorphism (RFLP), c) amplified fragment
length polymorphism (AFLP), and d) exon-primed, intron-crossing (EPIC)-PCR to identify or
differentiate fruit fly species especially sibling species within the B. dorsalis complex and genetic
variations between populations of a species.

INTERBREEDING BETWEEN B. dorsalis AND B. papayae

In laboratory and field cage experiments conducted towards the end of the last millennium, male B.
dorsalis (from Hawaii and Taiwan) mated readily with female B. papayae (Fig.1). Interbreeding also
occurred in a reciprocal cross. Inter-specific mating success was comparable to that of intra-specific
mating - between 59 and 78% of sexually mature females (50-150) mated in each replicate. Hybrids
up to F3 were successfully raised. The hybrid was as viable as the parental species (unpublished
data). Therefore, there is no hybrid sterility. This led us to suspect that the two species may be
strains or subspecies of a single species as their pheromonal systems are similar (especially the
booster pheromonal components are identical after methyl eugenol consumption – Tan and Nishida
1996); and to conduct simple DNA analysis to confirm or dispel the idea.

Recently, B. dorsalis and B. papayae have been shown to be not distinct genetic species, after DNA
analysis on intron alleles of the actin gene, but more of population differences between the two
(Haymer – see below). As such, it is not surprising that interbreeding of these “two species” occurred without any restrain. Presumably, in regions especially in Thailand where both these “species” are known to exist, there will be problem identifying specimens collected from the field. In addition, it has profound impact on areawide IPM and SIT, as well as in quarantine procedure - especially related to monitoring and security.

INTERBREEDING BETWEEN TWO SYMPATRIC SPECIES, B. carambolae AND B. papayae

B. carambolae is regarded as a distinct species from B. papayae based on morphological differences of a) wing costal band slightly enlarged at apex of Rs15, b) the presence of a dark spot on the fore femora, and c) shape and pattern of lateral black band on abdominal terga III-V (Drew and Hancock 1994); and differences in volatile components in the male rectal gland secretions (Perkins et al. 1990, Wee 2000). However, since 1992 males with intermediate morphological characters between the two species have surfaced among males captured in methyl eugenol traps during trapping in Penang Island (Tan 2000b). Are these males with intermediate morphological characters natural hybrids of the two sympatric species - B. carambolae and B. papayae? To answer this question confidently, a series of experiments were designed and conducted.

Copulatory Period and Hybrid Viability

B. carambolae and B. papayae have been known to mate from dusk to dawn. The actual time spent in copulation (CT) was compared for intra- and inter-specific mating conducted in the laboratory. Table 1 shows that mean CT for intra-specific mating for B. carambolae (680 min. = 11.3 h) is significantly longer than that of B. papayae (581 min. = 9.7 h). The mean CT for inter-specific mating is dependent on the female species involved. In a cross involving female B. carambolae (Fig.2) mean CT is not significantly different from that of intra-specific mating involving the conspecific female, but is significantly different with that of inter-specific mating involving B. papayae females - Table 1. This confirms that female fruit flies determine the copulatory period as females have been observed to kick vigorously to dislodge males to terminate copulation. In a related study conducted in Suriname, CT for inter-specific sterile B. dorsalis and B. carambolae was significantly lower (means in minutes ± standard error - from 4.67 ± 2.04 to 57.25 ± 19.15 and from 148 ± 143.01 to 271.50 for B. dorsalis male x B. carambolae female and B. carambolae male x B. dorsalis female crosses, respectively) than that for intra-specific cross for B. carambolae (387.38 ± 110.09 to 481.39 ± 49.84) (McInnis et al. 1999). The differences in results may be attributed to the differences in the strains of B. carambolae used.
Table 1  Mean* (+ standard error) copulatory period and eggs laid for intra- and inter-species mating of B. carambolae (BC) and B. papayae (BP)

<table>
<thead>
<tr>
<th>Type of mating</th>
<th>No. pairs</th>
<th>Cop. Period (min.)</th>
<th>Total eggs#</th>
<th>No. females</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP female x BP male</td>
<td>89</td>
<td>581.6 ± 22.9 a</td>
<td>91.0 ± 7.1 r</td>
<td>36</td>
</tr>
<tr>
<td>BP female x BC male</td>
<td>36</td>
<td>574.8 ± 36.3 a</td>
<td>36.3 ± 4.2 s</td>
<td>42</td>
</tr>
<tr>
<td>BC female x BP male</td>
<td>75</td>
<td>643.6 ± 13.1 b</td>
<td>46.5 ± 5.8 s</td>
<td>26</td>
</tr>
<tr>
<td>BC female x BC male</td>
<td>42</td>
<td>679.6 ± 15.1 b</td>
<td>23.1 ± 2.8 t</td>
<td>63</td>
</tr>
</tbody>
</table>

* Means with same alphabets within a column are not significantly different at P = 0.05.
# Total eggs laid per female in three oviposition days.
Adapted from Wee (2000).

The number of eggs laid in the first three oviposition attempts over different days by females of inter-species crosses is lower than that of B. papayae females but higher than that of B. carambolae females from intra-species crosses – Table 1. Egg hatchability (45-49%) was similar for females of intra-species crosses, but significantly lower for B. papayae females (20.2%) and higher for B. carambolae (75.5%) of inter-species crosses. On the contrary, pupation of larvae from intra- and inter-species crosses occurred at similar levels (90-94%), adult eclosion varies from 70 to 85%, and sex ratio of 1:1 were obtained from all intra- and inter-species crosses. The data show that interspecific mating between B. carambolae and B. papayae did not show any sign of hybrid sterility (Wee 2000).

Inter-Specific Mating in Outdoor Cage

In outdoor field cages, of 217 mating pairs observed, 17 (7.8%) were inter-specific mating between B. dorsalis and B. carambolae, of which 10 (4.6%) involved sterile B. dorsalis males (McInnis et al. 1999). In a male competition study, conducted in triplicates in a field cage (2.1 x 2.1 x 2.1 m^3) with 140-150 males of each species competing for the same number of females (either B. carambolae or B. papayae), B. papayae females showed a high preference for conspecific males (successful mating [mean ± standard error] with B. papayae males 95.3 ± 9.5, and with B. carambolae males 8.0 ± 4.6; P < 0.05); while, B. carambolae females showed no preference (successful mating with B. carambolae males 16.0 ± 2.1, and with B. papayae males 16.7 ± 5.2; P > 0.1) (Wee and Tan 2000). This work demonstrates that B. papayae female is more selective and prefers to mate with conspecific males; while B. carambolae female is not selective as regards her mate as no preference is shown to conspecific males. This non-selectivity in mate preference by B. carambolae females may account for natural interbreeding in the field as B. papayae males are more aggressive in mating behavior than B. carambolae males. Further, it may encourage the possibility of using sterile male—only B. dorsalis against wild B. carambolae in a SIT program if a higher percentage of inter-specific mating can be achieved. To achieve this, perhaps, one of the methods is to provide sources of methyl eugenol for the sterile males to feed on so as to boost its pheromonal system and, thus, mating performance (Tan and Nishida 1996).

Genitalia Length of Hybrid

Measurement of genitalia length of males and females was proposed as one of the methods to identify sibling species in the B. dorsalis complex (Iwaizumi et al. 1997). Based on aedeagal length, it was suggested that B. carambolae and B. papayae might have derived from B. dorsalis (Iwashashi 2000). It was reported that hybrids of B. carambolae and B. papayae have several intermediate characteristics, such as length of ovipositor and aedeagus (penis) of the two parental species since the early nineteen nineties (Tan 2000b). Table 2 shows that mean lengths of both aedeagus and aculeus of hybrid are intermediate in length between and are significantly different from their respective parental species. The average aculeus length for the parental species and hybrid is very similar to that determined by Khoo, S.G. (Tan 2000b). Based on these measurements, several wild males captured in Johor Baru, Johor and Relau, Penang possessed the intermediate aedeagal length (2.7-2.8 mm); and a female from Relau, Penang was found to have intermediate aculeus length (1.6-1.7 mm) (Wee 2000). It is inconclusive to infer that natural hybrids of B. carambolae and B.
papayae exist based solely on the intermediate morphological characters and genitalia length. As such, further procedures such as pheromonal component and DNA analyses need to be conducted to confirm that interbreeding occurred in the wild for the two sympatric sibling species.

Table 2  Mean* length (in mm) of male aedeagus and female aculeus of B. carambola (BC), B. papayae (BP) and F1 hybrid

<table>
<thead>
<tr>
<th>Species/hybrid</th>
<th>Aedeagus length (range)</th>
<th>n</th>
<th>Aculeus length (range)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. carambola</td>
<td>2.54a ± 0.09 (2.23 – 3.03)</td>
<td>20</td>
<td>1.44a ± 0.05 (1.35 – 1.50)</td>
<td>20</td>
</tr>
<tr>
<td>B. papayae</td>
<td>2.89b ± 0.09 (2.68 – 3.03)</td>
<td>20</td>
<td>1.82b ± 0.06 (1.73 – 1.93)</td>
<td>20</td>
</tr>
<tr>
<td>BP Female x BC Female</td>
<td>2.72c ± 0.10 (2.45 – 3.00)</td>
<td>39</td>
<td>1.64c ± 0.09 (1.40 – 1.83)</td>
<td>20</td>
</tr>
<tr>
<td>BC Male x BP Female</td>
<td>2.73c ± 0.08 (2.50 – 2.88)</td>
<td>43</td>
<td>1.60c ± 0.08 (1.50 – 1.80)</td>
<td>20</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation, and in parenthesis are minimum and maximum values; means with same alphabets within a column are not significantly different at P = 0.05. Adapted from Wee (2000).

Male pheromonal component of laboratory hybrid and detection of natural hybrid

Males of B. papayae produce trans coniferyl alcohol (CF) and 2-allyl-4,5-dimethoxyphenol (DMP) after consumption of methyl eugenol as pheromonal components (Nishida et al. 1988, Tan and Nishida 1996). Males of B. carambola convert methyl eugenol to CF. CF acts as a booster pheromonal component in addition to endogenous sex pheromonal components - N-3-methylbutyl acetamide (MBA) and 6-oxo-1-nonanol (OXO) at sexual maturity (Wee 2000). Hybrid males produce from a single component to a variety of combinations with endogenous components, after consuming methyl eugenol, as indicated in Table 3. Most F1 hybrid males (48-57%) contain a single detectable component – CF. 12-15% of the hybrid males possess a typical sex pheromone of B. papayae and 0-4% showed a typical sex pheromone of B. carambola – Table 3. However, 2-6% of the F1 hybrid males possess a combination of DMP (not detected in B. carambola) with MBA and/or OXO (endogenous components of B. carambola and not found in B. papayae) (Wee 2000).

Table 3  Percentage of hybrid of B. carambola (BC) X B. papayae (BP), after methyl eugenol consumption, with single or multiple male pheromonal components detected in male rectal gland

<table>
<thead>
<tr>
<th>Pheromonal component *</th>
<th>BC Female x BP Male n = 74</th>
<th>BP Female x BC Male n = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>57.5</td>
<td>48.0</td>
</tr>
<tr>
<td>DMP + CF (typical of B. papayae)</td>
<td>15.1</td>
<td>12.0</td>
</tr>
<tr>
<td>MBA + CF</td>
<td>12.3</td>
<td>36.0</td>
</tr>
<tr>
<td>OXO + CF</td>
<td>5.5</td>
<td>2.0</td>
</tr>
<tr>
<td>MBA + OXO + CF (typical of B. carambola)</td>
<td>4.1</td>
<td>0</td>
</tr>
<tr>
<td>MBA + DMP + CF</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>OXO + DMP + CF</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>MBA + OXO + DMP + CF</td>
<td>2.7</td>
<td>0</td>
</tr>
</tbody>
</table>

* CF = trans coniferyl alcohol; DMP = 2-allyl-4,5-dimethoxyphenol; MBA = N-3-methylbutyl acetamide; and OXO = 6-oxo-1-nonanol. Adapted from Wee (2000).

Based on a combination of components found only in typical B. carambolae and B. papayae males using GC-MS analysis, the detection of natural hybrids among captured wild males from different localities was conducted. Among the rectal glands analyzed from wild males captured, glands from two males (one from Air Itam, Penang and the other Batu Gajah, Perak – approximately 190 km apart) contained CF, DMP and MBA; and a further two (one from Tanjong Bungah, Penang and the other Johor Baru, Johor – about 700 km apart) possessed CF, DMP, MBA and OXO (Wee 2000). The latter natural hybrids which possess a combination of unique pheromonal components from both
parental species i.e. CF, DMP, MBA and OXO, show that interbreeding of *B. carambola* and *B. papayae* does occur in nature.

**DNA ANALYSIS OF FRUIT FLIES**

**RAPD-PCR Technique to Differentiate Endemic Pest Species**

Five endemic pest species in Malaysia – *B. albistragata*, *B. carambola*, *B. cucurbitae*, *B. latifrons* and *B. papayae*, were subjected to RAPD-PCR technique using four random oligonucleotide primers which yielded 34 molecular markers. Data matrix of Nei’s genetic distance (Table 4) were calculated from pair wise comparison of samples based on shared bands after PCR amplification. A dendrogram was then constructed using cluster analysis from the genetic distance data. It showed that the five species may be categorized into three groups – a) *B. latifrons* (not attracted to known lures), b) *B. albistragata* and *B. cucurbitae* (attracted to cue-lure/ raspberry ketone) and c) *B. carambola* and *B. papayae* (attracted to methyl eugenol) (Lim et al. 1995). The results indicated that RAPD-PCR technique accompanied by subsequent analyses is precise and efficient for distinguishing the endemic pest species.

**Table 4** Values for Nei’s distance of five endemic pest *Bactrocera* species* in Malaysia. – using RAPD-PCR technique

<table>
<thead>
<tr>
<th>Species</th>
<th>B. al</th>
<th>B. ca</th>
<th>B. cu</th>
<th>B. la</th>
<th>B. pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. al</td>
<td>0.0000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. ca</td>
<td>1.3195</td>
<td>0.0000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. cu</td>
<td>1.0782</td>
<td>2.2154</td>
<td>0.0000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. la</td>
<td>0.8524</td>
<td>1.4788</td>
<td>0.8320</td>
<td>0.0000</td>
<td>-</td>
</tr>
<tr>
<td>B. pa</td>
<td>1.5890</td>
<td>0.6060</td>
<td>0.8755</td>
<td>1.7483</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Adapted from Lim et al. (1995).

**Restriction Fragment Length Polymorphism (RFLP)**

This technique, with amplification of 18S plus complete Internal Transcribed Spacer 1 rDNA region of ribosomal DNA, using 21 restriction enzymes was developed for quarantine purposes. A core of nine restriction enzymes was selected to differentiate thirty eight species from five genera of tephritid fruit flies (Armstrong and Cameron 2000). This investigation successfully shows that most species, especially those from different genera of Tephritidae could be differentiated clearly and rapidly. But it has a limitation, the separation of sibling species in a complex is generally poor – none of the restriction enzymes produced clear diagnostic rDNA patterns between three sibling species of *B. dorsalis* complex - *B. dorsalis*, *B. papayae* and *B. philippinensis*. However, pair wise comparison of each of the sibling species against another sibling species *B. carambola*, showed clear diagnostic patterns for 7 restriction enzymes (Armstrong and Cameron 2000).

**AFLP Polymorphism of Amplified DNA Fragment**

AFLP was conducted for three sibling species *B. carambola*, *B. dorsalis* and *B. papayae* and a natural hybrid (captured from the wild, with intermediate morphological characters between *B. carambola* and *B. papayae*). Using two combinations of primers, a total of 264 amplified DNA fragments were detected in all the four samples. Of these fragments, 134 were monomorphic fragments (49.25% polymorphism). Within a range of 50 and 330 base pairs, 138 and 126 amplified DNA fragments were obtained from the primers – *E* ACC (5′-GACTGGCTACCAAATTCC ACC-3′) and *M* CAC/CAG (5′-GATGAGTCTGTGTAACACG/CAC-3′), respectively. The percentages detected polymorphism for primers combination *E* Acc/M CAC and *E* ACC/M CAG were 48.86 and 55.07, respectively. *B. carambola* showed the highest polymorphism (*E* ACC/M CAC = 29.71%; *E* ACC/M CAG =
followed by *B. dorsalis* (EACC/MCAG = 26.09%; EACC/MCAG = 23.08%), natural hybrid (EACC/MCAG = 26.09%; EACC/MCAG = 23.81%) and *B. papayae* (EACC/MCAG = 22.46%; EACC/MCAG = 17.46%). The natural hybrid sample showed similar polymorphism with that of *B. dorsalis*. This AFLP technique is a more sensitive technique than RAPD in differentiating closely related sibling species in a complex. However, in terms of cost, the former is approximately five times more than the latter.

From the calculated genetic distance (Table 5), *B. carambolae* appears to be isolated from the other two species and the natural hybrid (genetic distance 0.2596-0.2891). *B. dorsalis* is closer to the natural hybrid captured from the wild (genetic distance 0.0654) than to *B. papayae* (genetic distance 0.1303) (Lim 1999). This indicates that natural hybrid is more closely related to *B. dorsalis* than to *B. papayae*, and it is difficult to explain the result at this stage. Whatever proposed reason(s) will remain highly speculative until further research work is conducted on the natural hybrid in relation to existing populations of *B. carambolae* and *B. papayae* throughout the region. It should be noted that *B. dorsalis* and *B. papayae* are not distinct genetic species (Haymer, personal communication) and *B. dorsalis* does not exist in Malaysia (Drew and Hancock 1994).

Table 5 Data matrix of Nei’s genetic distance for *B. carambolae*, *B. dorsalis* and *B. papayae* and natural hybrid (*B. carambolae* X *B. papayae*) - using AFLP-PCR technique

<table>
<thead>
<tr>
<th>Species/hybrid</th>
<th>B. carambolae</th>
<th>B. papayae</th>
<th>Hybrid</th>
<th>B. dorsalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. carambolae</td>
<td>0.0000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. papayae</td>
<td>0.2891</td>
<td>0.0000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hybrid</td>
<td>0.2841</td>
<td>0.1390</td>
<td>0.0654</td>
<td>0.0000</td>
</tr>
<tr>
<td>B. dorsalis</td>
<td>0.2596</td>
<td>0.1303</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Adapted from Lim (1999).

Amplification of Transposon Element Using the Polymerase Chain Reaction (PCR)

Amplification of *hobo*-related element from genomic DNA using the PCR was performed for wild-type and mutant strains of five species, *Anastrepha suspensa*, *B. cucurbitae*, *B. dorsalis*, *Ceratitis capitata* and *Toxotrypana curvicauda*, representing four genera of tephritid fruit flies. Sequences of *hobo*-related element were identified in all species except *T. curvicauda* (Handler and Gomez 1996). All of the sequences exist as multiple genomic elements; and a deleted form of the *B. cucurbitae* element exists in *B. dorsalis*. Further, two *hobo*-related elements were found in *B. dorsalis*; and Handler and Gomez (1996) suggested that *Bd-HRE* (distantly related to *hobo* in *B. dorsalis*) may have evolved vertically within the species, while *Be-HRE*, (more closely related to *hobo* in *B. cucurbitae*) may have originated from a more recent horizontal transfer.

Using specific pairs of *sense* and *antisense* degenerate oligonucleotide primers to amplify transposon elements, the *hobo*-like and *mariner*-like elements in different *B. papayae* populations were successfully amplified. A 454 base pair fragment of *hobo*-like transposon element was amplified in a population that originated in Tanjung Bunga, Penang Island. However, *mariner*-like transposon element fragment of 495 base pair was amplified from a population in Juru, Seberang Prai in the mainland of Malaysia (Lim 1999). It was clear that both the transposon elements did not occur in any single population. Therefore, the products of PCR amplification of the *hobo*-like and *mariner*-like transposon elements are important in further studies of genetic variability and population evolution of *B. papayae*.

Exon Primed, Intron Crossing PCR (EPIC-PCR)

This method uses primers designed from conserved exon regions of nuclear genes for the amplification of intron sequences of selected genes (Palumbi and Baker 1994). Using this technique in a recent investigation by He and Haymer (1997) on *B. dorsalis* populations, primers were designed using exon sequences from the *Bd41* actin gene. The sequences of the forward (designated BdA1F1) and backward (designated BdA1B1) primers are 5'-CTTGGGCGTGGAAATCGTTG-3'.
and 5'-TTGATGGTGAAGATTTCAG-3', respectively (He and Haymer 1994). The PCR and EPIC primers were used to amplify the intron sequences (intervening sequences or noncoding sequences that tend to be variable within and between species) of the actin gene from individuals representing three different wild B. dorsalis populations and two different laboratory B. dorsalis populations. Three alleles were identified by direct sequencing (Table 6) and they exist in different frequencies in the populations studied. Allele 1 (BdorA1) exists at different frequencies in all three wild populations (Kauai, Molokai, in Hawaii and Thailand), allele 2 (BdorA2) exists only in the Hawaiian populations and allele 3 exists only in the population from Thailand. This study suggests that intron sequences can provide a high resolution picture of the genetic makeup of populations within B. dorsalis, and also the ability to define precisely all the existing allelic variations which may be useful in differentiating sibling species in a population (He and Haymer 1997). Haymer and his team in the University of Hawaii at Manoa, have extended the EPIC-PCR investigation on intron sequences of the actin gene to B. carambolae and B. papayae populations from Malaysia. They found that B. carambolae has two alleles BcArA1 and BcArA2 with 3 and 23 nucleotide deletions when aligned with allele BdorA1 of B. dorsalis; and B. papayae has three alleles - BpapA1, BpapA2 and BpapA3 (Table 6). A comparison of alleles between B. dorsalis and B. papayae shows allele BpapA2 is identical to allele BdorA1. This indicates that B. dorsalis and B. papayae share a common allele as part of their genetic makeup. Therefore, it suggests that B. dorsalis and B. papayae are not genetically distinct species and that the variations observed are consistent with that of population differences. In an allelic comparison with B. carambolae, the alleles show relatively few single nucleotide changes, but the major difference is the extensive deletion in the BcarA2 allele. Since the large deletion observed is contiguous, it most likely represents a single event equivalent to a single base change (Haymer - personal communication). The use of DNA-based analytical techniques, in comparison with allozyme/isozyme techniques, has revealed much higher levels of polymorphisms, and, therefore, a more precise picture of the true genetic structures of insect populations (He and Haymer 1997).

CONCLUSION

Bactrocera dorsalis and B. papayae interbreed readily and produce viable offspring under laboratory conditions. Under laboratory observation of B. carambolae and B. papayae interbreeding, the average number of eggs laid by hybrid females was lower than that of B. papayae females but higher than that of B. carambolae females of intra-specific crosses. For inter- and intra-specific mating, the copulatory period is dependent on the female species involved – female B. carambolae copulates significantly longer than that of B. papayae female. Aedeagal and aculeus lengths of hybrids are intermediate between those of their respective parental species. Hybrid males have one to four sex pheromonal components after consumption of methyl eugenol; 2 - 6 % of them possess a combination of endogenous pheromonal components specific to B. carambolae and components derived from methyl eugenol typical of B. papayae. Based on the latter, four wild males captured from different parts of Peninsular Malaysia possessed combination of the sex pheromonal components.

DNA analysis using PCR techniques was very useful in differentiating pest species. Using AFLP polymorphism of amplified DNA fragment plus calculated NeI’s genetic distance showed that natural hybrid of B. carambolae and B. papayae was closer to B. dorsalis than to the parental species. Using exon primed, intron crossing PCR, one of the three alleles of actin gene intron of B. dorsalis has identical DNA sequence to one of three allelic introns of the same gene in B. papayae which suggests that the two species are not distinct genetic species.

A Hobo-like transposon element was detected in a population from Penang Island, while in a population from the mainland of Peninsular Malaysia, a mariner-like transposon element was detected.
Table 6. Alignment of Intron allele nucleotide sequences of actin gene (BdAl) in Bactrocera
dorsalis, B. papayae, B. carambola.*

<table>
<thead>
<tr>
<th>Species and allele</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>B. dorsalis</strong></td>
<td></td>
</tr>
<tr>
<td>BdozA1</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AAATAATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC TTTTTTTATA TCTTTACAG</td>
</tr>
<tr>
<td>BdozA2</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AAATAATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC TTTTTTTATA TCTTTACAG</td>
</tr>
<tr>
<td>BdozA3</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AAATAATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC TTTTTTTATA TCTTTACAG</td>
</tr>
<tr>
<td><strong>B. papayae</strong></td>
<td></td>
</tr>
<tr>
<td>BpapA1</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AGAGATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC TTTTTTTATA TCTTTACAG</td>
</tr>
<tr>
<td>BpapA2</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AAATAATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC TTTTTTTATA TCTTTACAG</td>
</tr>
<tr>
<td>BpapA3</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AAATAATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC TTTTTTTATA TACTTTAC</td>
</tr>
<tr>
<td><strong>B. carambola</strong></td>
<td></td>
</tr>
<tr>
<td>BcarA1</td>
<td>GTAATTTAAT TGTTCCACA AAAAGCCAG AAATAATGAA TAAAGCCTTT GCCTGTTTT CGATGATTC AACGAT... TTTTTTTATA TCTTTACAG</td>
</tr>
<tr>
<td>BcarA2</td>
<td>GTAATTTAAT TGTTCC... .......... .......... A TAAAGCCTTT GCCTGTTTT CGATGATTC AACGGAATTC .TTTTTTATA TCTTTACAG</td>
</tr>
</tbody>
</table>

Nucleotide base.... A = Adenine; C = Cytidine; G = Guanine; and T = Thymine.

**Note:** 1) Changes in each allele are indicated by A, C, G, or T replacing an original nucleotide base in BdozA1; 2) A "." indicates a nucleotide has been deleted; and 3) For B. carambola, 3 and 23 nucleotides were deleted in BcarA1 and BcarA2, respectively. *

*Adapted from D. Haymer, M. He and C. Naole (personal communication).*
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REFERENCE


